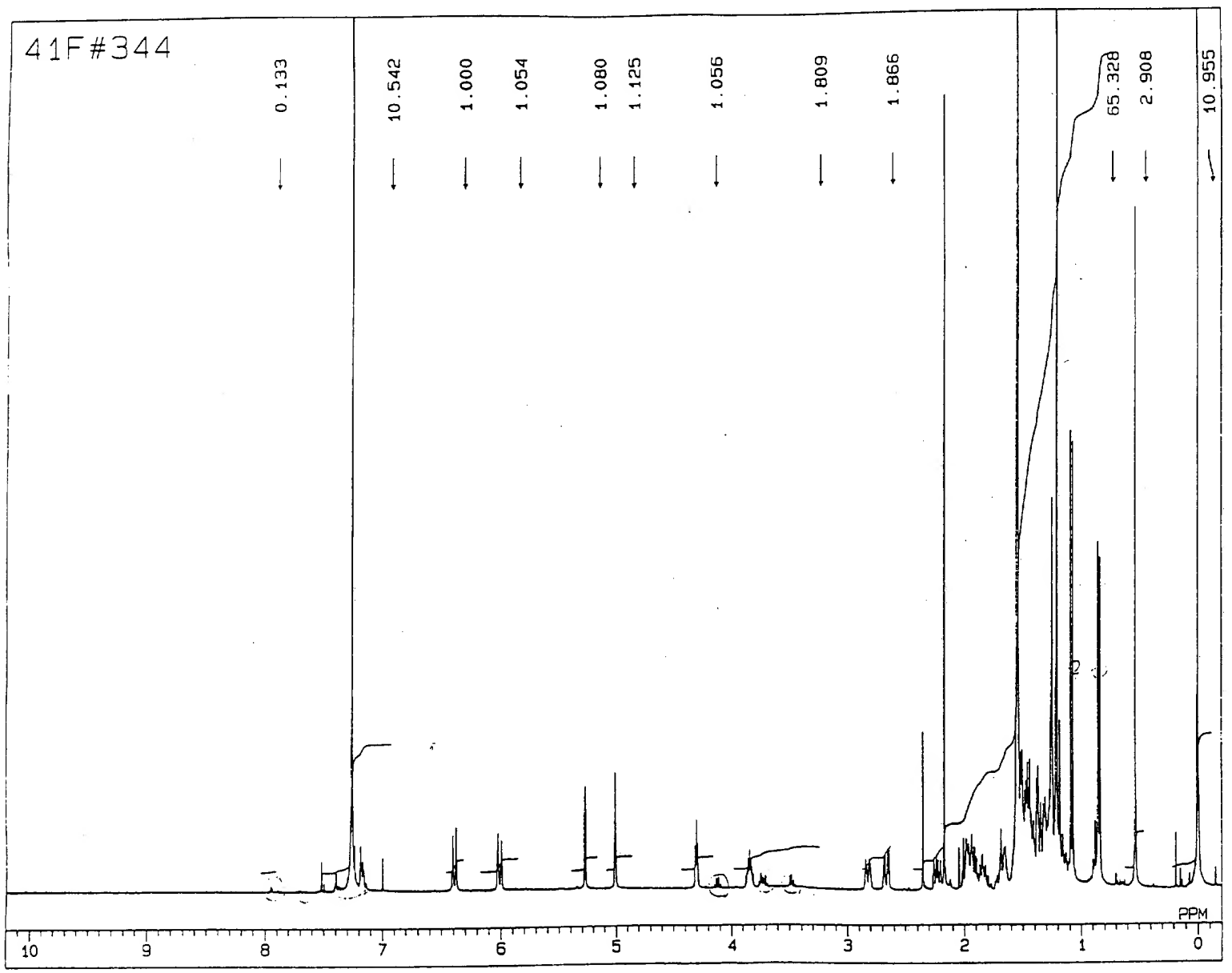
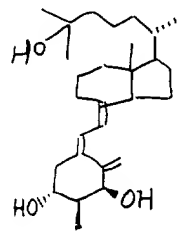
10: 58: 02




```
SLVNT  CDCL3
OBNUC  1H
OBFRQ        399.65 MHz
OBSET        124.00 kHz
OBFIN       10905.1 Hz
PW1             5.9 us
POINT         32768
SAMPO         32768
SCANS          9216
DUMMY             0
FREQU        5000.0 Hz
FILTR          5000 Hz
ACQTM         3.277 sec
PD            5.000 sec
RGAIN    24
BF              0.10 Hz
T1        0.0 %
T2        0.0 %
T3       90.0 %
T4      100.0 %
EXMOD  SGNON
DFILE  [100, 140]FN0344
SHMFL  TH5
SPEED            15 Hz
OPERATOR     J.SHIMODE
```



PEAK	173	
MXINT	996134500	
RESOL	0.3100000	Hz
RESOL	0.0007754	ppm
EXREF	0.0000000	ppm
OBS	-2398.68	Hz
ABOBS	399784.9000000	KHz
NGAIN	22	
COMNT	41F#344	

NO.	PPM	INT(%)	FREQ(Hz)	POSITION	BAR GRAPH
1	7.51981	0.51055	3006.29	6201	
2	7.49843	0.13021	2997.74	6229	
3	7.40836	0.12475	2961.73	6347	
4	7.40530	0.13811	2960.51	6351	
5	7.40225	0.14221	2959.29	6355	
6	7.39614	0.26281	2956.85	6363	
7	7.39385	0.30828	2955.93	6366	
8	7.27401	0.81013	2908.02	6523	
9	7.26103	100.00000	2902.83	6540	++++++++++++++++++++
10	7.24347	0.52661	2895.81	6563	
11	7.23813	0.77035	2893.68	6570	
12	7.18469	0.76414	2872.31	6640	
13	7.17782	0.42273	2869.57	6649	
14	7.16790	0.44624	2865.60	6662	
15	7.16637	0.50551	2864.99	6664	
16	7.16485	0.47254	2864.38	6666	
17	7.15798	0.35221	2861.63	6675	
18	7.14042	0.13947	2854.61	6698	
19	6.99691	0.56436	2797.24	6886	
20	6.40531	0.93125	2560.73	7661	
21	6.37706	1.07192	2549.44	7698	
22	6.02516	0.95532	2408.75	8159	
23	5.99691	0.83247	2397.46	8196	
24	5.28089	1.51583	2111.21	9134	
25	5.27860	1.69251	2110.29	9137	
26	5.27554	1.73099	2109.07	9141	
27	5.27325	1.62102	2108.15	9144	
28	5.01677	0.75333	2005.62	9480	
29	5.01142	1.94912	2003.48	9487	
30	5.00684	1.89288	2001.65	9493	
31	4.31372	0.71248	1724.55	10401	
32	4.30303	1.15135	1720.28	10415	
33	4.29311	0.75016	1716.31	10428	
34	4.13051	0.16040	1651.31	10641	
35	4.11295	0.17989	1644.29	10664	
36	3.87631	0.20966	1549.68	10974	
37	3.86334	0.33502	1544.49	10991	
38	3.85647	0.49772	1541.75	11000	
39	3.85265	0.38111	1540.22	11005	
40	3.84425	0.63931	1536.87	11016	
41	3.83662	0.39411	1533.81	11026	
42	3.83280	0.51341	1532.29	11031	
43	3.82517	0.36634	1529.24	11041	
44	3.81296	0.23818	1524.35	11057	
45	3.76334	0.10740	1504.52	11122	
46	3.75723	0.10787	1502.08	11130	
47	3.74654	0.24683	1497.80	11144	
48	3.74425	0.15079	1496.89	11147	
49	3.73891	0.18680	1494.75	11154	
50	3.72975	0.13429	1491.09	11166	
51	3.72593	0.16958	1489.56	11171	
52	3.72135	0.16516	1487.73	11177	
53	3.70838	0.15702	1482.54	11194	
54	3.70532	0.20963	1481.32	11198	
55	3.49922	0.21425	1398.93	11468	
56	3.48777	0.15752	1394.35	11483	
57	3.48548	0.22505	1393.43	11486	
58	3.47097	0.13086	1387.63	11505	
59	2.84502	0.48178	1137.39	12325	
60	2.83518	0.35204	1133.42	12338	
61	2.81372	0.48347	1124.88	12366	
62	2.80609	0.41017	1121.83	12376	
63	2.69185	0.53931	1076.05	12526	
64	2.68166	0.56430	1072.08	12539	
65	2.65800	0.65372	1062.62	12570	
66	2.64808	0.63403	1058.65	12583	
67	2.35571	2.61120	941.77	12966	+
68	2.25877	0.50723	903.02	13093	
69	2.23892	0.54138	895.08	13119	
70	2.22518	0.46140	889.59	13137	
71	2.20534	0.45036	881.65	13163	
72	2.17251	13.00911	868.53	13206	+++
73	2.12365	0.13311	849.00	13270	
74	2.04579	0.66131	817.87	13372	
75	2.00915	0.82413	803.22	13420	
76	1.98396	0.81436	793.15	13453	
77	1.97403	0.63916	789.18	13466	
78	1.96564	0.72810	785.83	13477	
79	1.93892	0.88387	775.15	13512	
80	1.92976	0.56873	771.48	13524	
82	1.91220	0.65209	764.47	13547	
83	1.90304	0.51956	760.80	13559	
84	1.89388	0.47665	757.14	13571	
85	1.88625	0.22313	754.09	13581	
86	1.87633	0.21354	750.12	13594	
87	1.86869	0.37384	747.07	13604	
88	1.85877	0.31288	743.10	13617	
89	1.85342	0.45550	740.97	13624	
90	1.84884	0.54529	739.14	13630	
91	1.84274	0.35213	736.69	13638	
92	1.83663	0.33970	734.25	13646	
93	1.82823	0.30126	730.90	13657	
94	1.81984	0.36071	727.54	13668	
95	1.79770	0.18736	718.69	13697	
96	1.71907	0.19656	687.26	13800	
97	1.68854	0.97399	675.05	13840	
98	1.67327	0.31122	668.95	13860	
99	1.66259	0.65576	664.67	13874	
100	1.65190	0.67322	660.40	13888	
101	1.55266	24.74699	620.73	14018	+++++
102	1.54961	23.68475	619.51	14022	+++++
103	1.52289	2.25819	608.83	14057	
104	1.51908	2.14206	607.30	14062	
105	1.50915	2.30518	603.33	14075	
106	1.50534	2.03049	601.81	14080	
107	1.49694	1.34147	598.45	14091	
108	1.48778	1.63270	594.79	14103	
109	1.47785	1.78724	590.82	14116	
110	1.46488	2.09681	585.63	14133	
111	1.45572	1.46173	581.97	14145	
112	1.44885	1.68220	579.22	14154	
113	1.44350	2.15006	577.09	14161	
114	1.43816	1.24164	574.95	14168	
115	1.43129	1.03275	572.20	14177	
116	1.42137	0.99580	568.24	14190	
117	1.41297	1.10303	564.88	14201	
118	1.40610	0.79794	562.13	14210	
119	1.39770	0.64862	558.78	14221	
120	1.38549	1.82600	553.89	14237	
121	1.38167	1.78330	552.37	14242	
122	1.37327	2.04219	549.01	14253	
123	1.35037	1.41708	539.86	14283	
124	1.34274	0.83533	536.80	14293	
125	1.32747	1.39920	530.70	14313	
126	1.31984	1.38789	527.65	14323	
127	1.31144	1.51146	524.29	14334	
128	1.29847	1.01536	519.10	14351	
129	1.29312	1.05290	516.97	14358	
130	1.27786	1.50170	510.86	14378	
131	1.27404	1.64288	509.34	14383	
132	1.26259	2.70069	504.76	14398	+
133	1.25419	6.42871	501.40	14409	+
134	1.24503	2.32149	497.74	14421	
135	1.24198	1.52839	496.52	14425	
136	1.23053	1.11573	491.94	14440	
137	1.22747	1.52209	490.72	14444	
138	1.21373	32.96595	485.23	14462	+++++++
139	1.18778	2.79381	474.85	14496	+
140	1.16946	0.62663	467.53	14520	
141	1.16030	0.87387	463.87	14532	
142	1.14274	0.51726	456.85	14555	
143	1.13893	0.53558	455.32	14560	
144	1.13434	0.55867	453.49	14566	
145	1.12442	0.43039	449.52	14579	
146	1.10763	0.40549	442.81	14601	
147	1.09160	7.52555	436.40	14622	++
148	1.07480	7.34448	429.69	14644	+
149	1.05572	0.30532	422.06	14669	
150	1.00915	0.11803	403.44	14730	
151	0.93893	0.11868	375.37	14822	
152	0.93206	0.12279	372.62	14831	
153	0.92061	0.16872	368.04	14846	
154	0.91450	0.15377	365.60	14854	
155	0.89618	0.45087	358.28	14878	
156	0.89236	0.36908	356.75	14883	
157	0.88015	1.10219	351.87	14899	
158	0.86183	0.92495	344.54	14923	
159	0.85572	5.69947	342.10	14931	+
160	0.83893	5.44266	335.39	14953	+
161	0.69923	0.21725	279.54	15136	
162	0.66488	0.10733	265.81	15181	
163	0.62442	0.10717	249.63	15234	
164	0.53282	11.16377	213.01	15354	++
165	0.18321	0.88836	73.24	15812	
166	0.14580	0.29633	58.29	15861	
167	0.06870	0.21470	27.47	15962	
168	0.00763	3.17474	3.05	16042	+
169	0.00000	83.23671	0.00	16052	+++++++++++++++++
170	-0.00840	3.40815	-3.36	16063	+
171	-0.03893	0.15635	-15.56	16103	
172	-0.04427	0.14362	-17.70	16110	

Experimental note of VDR binding affinity with English translation
Compound (68) / 20epi Aa / # 346 and Compound (72) / 20epi Ds / # 344

Experiment of Bovine Thymus VDR binding affinity (# 7)

① Make phosphate-potassium buffer Keeping at 4℃

② Diluted solution series of 1α,25(OH)2D3, #344, #346

③ Concentration preparation of [26,27-methyl3H] 1α,25(OH)2D3 solution
Take 100 μL and evaporate Add 6.25 mL of Japanese pharmacopeia grade ethanol

④ Pour sample / 50 μL Japanese pharmacopeia grade ethanol (②) into disposable culture tube (12 x 75 mm IWAKI) in concentration order (from thin to dense)
(like ⑭ ㉘ →①⑮)
⑧⑤ → ⑨⑥are Japanese pharmacopeia grade ethanol only (by dispenser)

⑤ Make receptor solution (lot 110431 YAMASA)
Pour 5 mL of phosphate-potassium buffer (①) into a vessel containing thymus receptor and dissolve the receptor gently. Add further 50 mL of the buffer and stir gently

⑥ Add 500 μL of the receptor solution to each tubes except blank (⑧⑨ ⑨⓪ ⑨① ⑨②)
Add 500 μL of the buffer solution to each blank tube

⑦ Stir by vortex, avoid forming

⑧ Pre incubate at rt for 1 hr
Put the top on the tubes by plastic wrap & aluminum foil
13:40 ～ 14:40 rt approximately 22℃

RI room

⑨ Add 50 μL of the hot solution (③) to each tubes by dispenser
In case of hot only count (97 98 99 100), hot solution is added to vial tube

⑩ Stir by vortex, avoid forming

⑪ Put the top on the tubes by plastic wrap, put the tubes into 4℃ refrigerator in RI room, and stand overnight 15:10～

97	16217.7 dpm	
98	16349.9	
99	16280.0	
100	16634.8	
101	54.3	
102	28.3	
103	42.7	
104	56.9	Average 16370 dpm
		" 45 dpm

Add 10 mL of ACS-II and measure radioactivity count for 1 min by Aloka A
Stand rt and measure radioactivity count for 2 min tomorrow

(16370 dpm = 273 dps = 273 Bq
11.4 GBq / mg therefore 24 pg / tube)

~9:25

⑫ Put out the yesterday's samples from the refrigerator in RI room and add 200 μL of DCC solution (lot M602 YAMASA) to each tubes by dispenser except total count tubes (㉓ ㉔ ㉕ ㉖)

Add the buffer solution ① to each total count tubes

⑬ Vortex tubes

⑭ Stand for 30 min at 4℃ 9:50~10:20

10:30~10:40

⑮ Centrifuge at 3000 rpm for 10 min at 0℃

⑯ Transfer 500 μL of supernatant to 20 mL WHEATON vial

Lay ice on tray and put tube on the ice

in concentration order (from thin to dense) ①→⑭ same pipetter tip

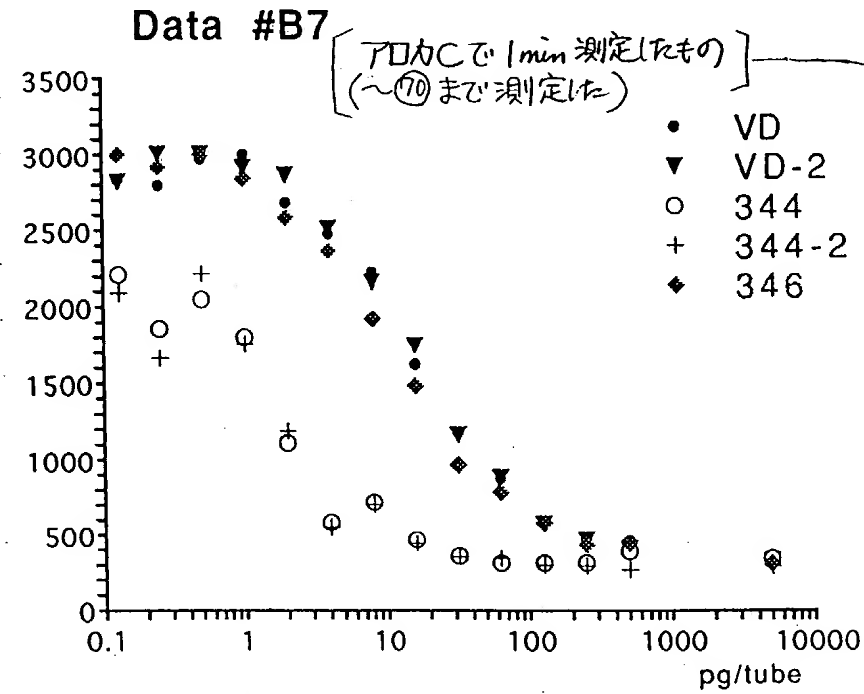
Change pipetter tip ⑮→㉘

⑰ Add 9.5 mL of ACS-II to each tubes, shake, and measure radioactivity count (2 min)

Aloka A

☆ バッファ
☆ バイアル
☆ 分注器
☆ ピペットマン 1000 200
☆ 〃
☆ レレレ-ズ
☆ チップたくさん

No. year month day ( )



[This shows the results of 1 min measuring by Aloka C (measured to ～⑦⓪)]

Exhibit 1
Note 3, p. 4
(Translation)

	L3		オレンジ		みどり	
/50µl	1α25(OH)2 VD3		#344		#346	
5ng	[1] 290	[15] 325	[29] 308	[43] 296	[57] 338	[71] 305
500pg	[2] 357	[16] 363	[30] 325	[44] 312	[58] 445	[72] 386
250	[3] 444	[17] 529	[31] 318	[45] 302	[59] 445	[73] 477
125	[4] 608	[18] 623	[32] 326	[46] 324	[60] 528	[74] 573
63	[5] 802	[19] 806	[33] 349	[47] 326	[61] 698	[75] 623
32	[6] 1094	[20] 1166	[34] 391	[48] 387	[62] 1041	[76] 913
16	[7] 1701	[21] 1676	[35] 458	[49] 369	[63] 1395	[77] 1357
8	[8] 2164	[22] 2109	[36] 658	[50] 663	[64] 1834	[78] 1822
4	[9] 2494	[23] 2511	[37] 568	[51] 520	[65] 2428	[79] 2180
2	[10] 2519	[24] 2536	[38] 1145	[52] 1161	[66] 2766	[80] 2499
1	[11] 2879	[25] 2768	[39] 1739	[53] 1819	[67] 2768	[81] 2763
0.5	[12] 2862	[26] 2924	[40] 2081	[54] 2062	[68] 2762	[82] 2768
0.25	[13] 2851	[27] 2959	[41] 1942	[55] 1847	[69] 2910	[83] 2834
0.13	[14] 2839	[28] 2690	[42] 1987	[56] 1932	[70] 2990	[84] 2694

MR series

0	85 2744	86 2982	87 3149	88 3048	2980
blank	89 224	90 166	91 174	92 311	218
totalcount	93 7965	94 8280	95 8052	96 8325	8155
[入れた量]	97 16184	98 15926	99 16360	100 16561	16257
blank	101 27	102 59	103 43	104 34	40

{ added amount }

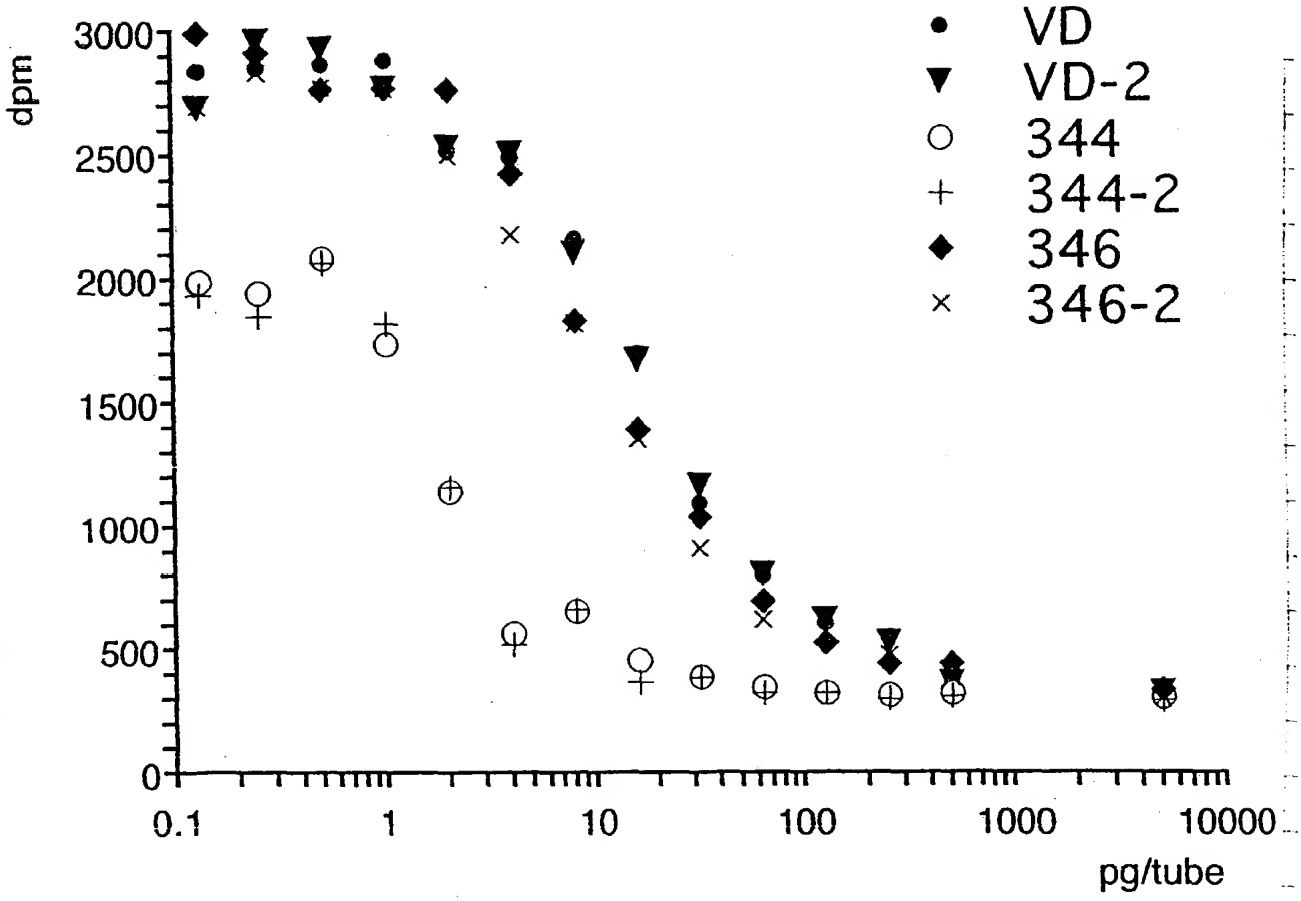
(2762)

[すべての実験値から218をひいて (2980−218)で割り ×100して Bound[%]をだします]

{ Bound[%] was calculated as follows: Subtract 218 from all experimental values, then this value divides by (2980 - 218) and multiply 100. }

50/(50+500+200)

#B7



	pg/tube	VD	VD-2	344	344-2	346	346-2
0	5000.0	290.00	325.00	308.000	296.00	338.00	305.00
1	500.00	357.00	363.00	325.000	312.00	445.00	386.00
2	250.00	444.00	529.00	318.000	302.00	445.00	477.00
3	125.00	608.00	623.00	326.000	324.00	528.00	573.00
4	63.000	802.00	806.00	349.000	326.00	698.00	623.00
5	32.000	1094.0	1166.0	391.000	387.00	1041.0	913.00
6	16.000	1701.0	1676.0	458.000	369.00	1395.0	1357.0
7	8.0000	2164.0	2109.0	658.000	663.00	1834.0	1822.0
8	4.0000	2494.0	2511.0	568.000	520.00	2428.0	2180.0
9	2.0000	2519.0	2536.0	1145.00	1161.0	2766.0	2499.0
10	1.0000	2879.0	2768.0	1739.00	1819.0	2768.0	2763.0
11	0.50000	2862.0	2924.0	2081.00	2062.0	2762.0	2768.0
12	0.25000	2851.0	2959.0	1942.00	1847.0	2910.0	2834.0
13	0.13000	2839.0	2690.0	1987.00	1932.0	2990.0	2694.0

dpm

<Results>

blank = 224 + 166 + 174 + 311 / 4 = 218

0 = 2744 + 2982 + 3149 + 3048 / 4 = 2980

Bound[%] was calculated as follows: Subtract 218 which is average value of blank from all experimental values, then this value divides by (subtract 218 from 2980 which is average value of drug 0)(2980 – 218 = 2762) and multiply 100

total count = 7965 + 8280 + 8052 + 8325 / 4 = 8155 dpm

8155 / 60 dps = 136 Bq As I put 500 μL from 800 μL and measured radioactivity count

136 x 8 / 5 = 217 Bq

11.4 GBq / mg therefore 19 pg / tube

As average added amount is 16257 dpm

from 271 Bq

24 pg / tube

Approximately 80% of hot receptor exists in solution

and the rest should absorb an inside wall of glass tube

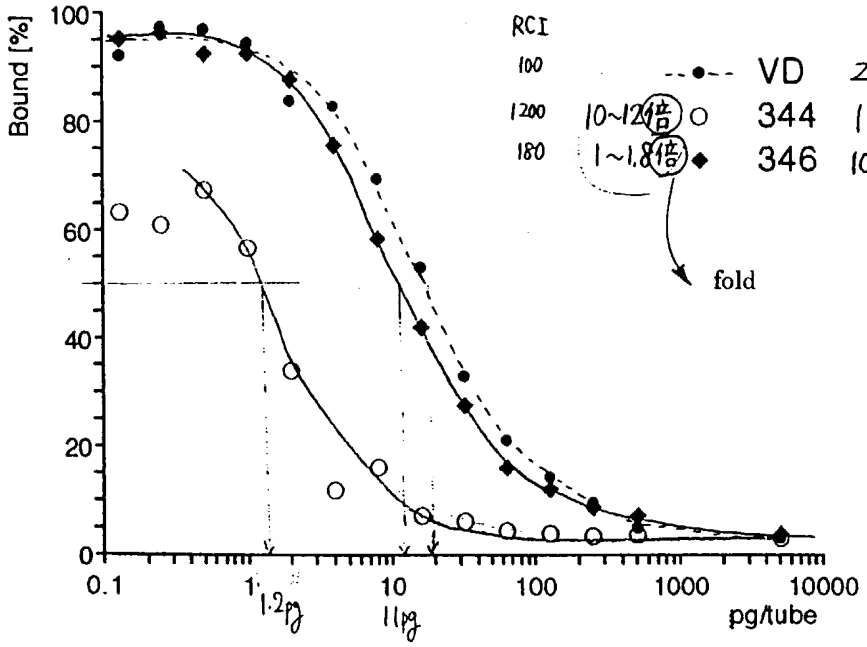
217 Bq / tube = 217 / 4.85T / (50 + 500 + 50) μL

= 0.075 nM

Or, it may exists as 1α25(OH)2 and the rest may count of decompose stuff

Bovine
Chicken

#B7(edit)



pg/tube	VD	VD-2	VD-	344	344-2	344-	346	346-2	346-
5000.0	2.6068	3.8740	3.2404	3.25851	2.8240	3.0413	4.3447	3.1499	3.7473
500.00	5.0326	5.2498	5.1412	3.87400	3.4033	3.6387	8.2187	6.0825	7.1506
250.00	8.1825	11.260	9.7212	3.62056	3.0413	3.3309	8.2187	9.3773	8.7980
125.00	14.120	14.663	14.392	3.91021	3.8378	3.8740	11.224	12.853	12.038
63.000	21.144	21.289	21.217	4.74294	3.9102	4.3266	17.379	14.663	16.021
32.000	31.716	34.323	33.020	6.26358	6.1188	6.1912	29.797	25.163	27.480
16.000	53.693	52.788	53.240	8.68936	5.4671	7.0782	42.614	41.238	41.926
8.0000	70.456	68.465	69.461	15.9305	16.112	16.021	58.508	58.074	58.291
4.0000	82.404	83.020	82.712	12.6720	10.934	11.803	80.014	71.035	75.525
2.0000	83.309	83.925	83.617	33.5626	34.142	33.852	92.252	82.585	87.419
1.0000	96.343	92.324	94.334	55.0688	57.965	56.517	92.324	92.143	92.234
0.50000	95.728	97.972	96.850	67.4511	66.763	67.107	92.107	92.324	92.216
0.25000	95.329	99.240	97.285	62.4185	58.979	60.699	97.466	94.714	96.090
0.13000	94.895	89.500	92.198	64.0478	62.056	63.052	100.36	89.645	95.004

Bovine Thymus VDRへの結合実験（#7）

① リン酸カリバッファを作製　4℃保存
② 1α25(OH)2VD3, #344, #346の希釈系列
③ [26,27-methyl 3H] 1α,25(OH)2VD3の濃度調製
100μlとってとばし　6.25mlの局エタ

④ disposable culture tube (12×75mm イワキ)に
sample / 50μl局エタ(②)をうすい順にいれていく
（⑭⑮→①⑮のように）
⑧⑨→⑯は局エタのみ.（分注器で）

⑤ レセプタ溶液をつくる（lot 11043 ヤマサ）
Thymus Receptorの容器にリン酸カリバッファ①を
5ml加えて静かにとかす　さらに50mlを
加え静かにまぜる.

⑥ レセプタ溶液500μlを　blank（⑧⑨⑪⑫）
以外のtubeに加える
加えなかったtubeには　bufferを500μl加える

⑦ vortexであわだてないようにかくはんする

⑧ rtで1hr pre incubation
ラップ&ホイルでふた　rt 22℃ぐらい
13:40～14:40

#

RI室

⑨ hot溶液(③)をすべてのtubeに分注器で50μlずつ加える.
hotのみcount(97 98 99 100)にはバイアルに入れる.

⑩ vortexでおわだてないようにかくはんする

⑪ ラップでふたをして 4℃のRI室の冷蔵庫に入れ over night. 15:10〜

97	16217.7 dpm
98	16349.9
99	16280.0
100	16634.8
101	54.3
102	28.3
103	42.7
104	56.9

平均 16370 dpm
± 45 dpm

10mlのACS-IIを加えてアロカAで1min countする.
RTで放置し次の日にいっしょに2min count.

(16370 dpm = 273 dps = 273 Bq
11.4 GBq / mg だから 24 pg/tube)

遠心 0℃ スイッチon

～9:25 RI室の

⑫ 前日のサンプルを冷蔵庫から出して total count (⑬⑭⑮⑯) 以外の tube に DCC液を (lot M602 ヤマサ) 200μl ずつ 分注器でかえる
かえなかった tube には ①のバッファをかえる

⑬ tube を vortex

⑭ 4℃で 30min 放置 9:50～10:20

10:30～10:40

⑮ 遠心 0℃ 10min 3000rpm

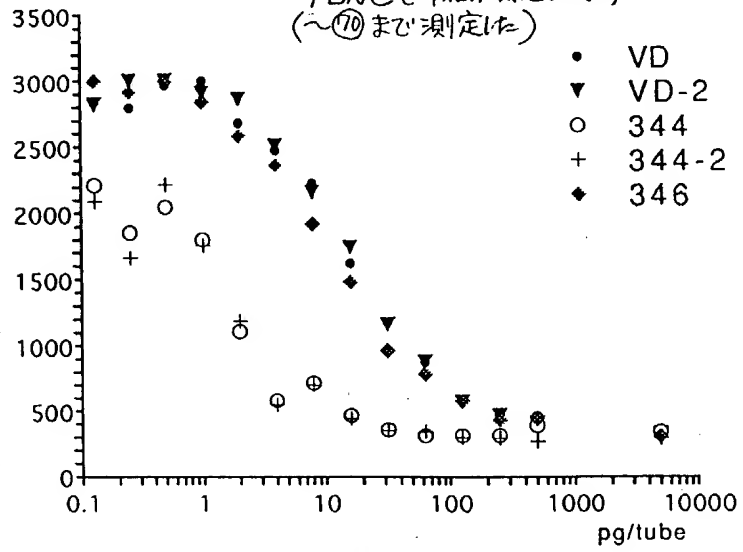
⑯ 上澄を 500μl ずつ WHEATON の 20ml の バイアルに 移す バットの上に氷をしいてしけんかん たてをならべた
(うすい順に ①→⑭ チップ同じ
チップかえて ⑮→㉘

⑰ ACS-II を 9.5ml ずつかえて shake し count (2min) する アロカA

☆ ﾊﾝﾄﾘｱ　　☆ ﾚﾚｰﾊﾞ
☆ バイアル　　☆ チップたくさん
☆ ﾉｶ注ｷｶﾞ
☆ ﾓﾍﾟｯﾄﾏﾝ1000 200
☆ 〃

Data #B7

アロカCで1min測定したもの
(～⑦⓪まで測定した)



L3 オレンジ みどり

/50μl	1α,25(OH)$_2$VD3		#344		#346	
5ng	290	325	308	296	338	305
500pg	357	363	325	312	445	386
250	444	529	318	302	445	479
125	608	623	326	324	528	573
63	802	806	349	326	698	623
32	1094	1166	391	387	1041	913
16	1701	1676	458	369	1395	1357
8	2164	2109	658	663	1834	1822
4	2494	2511	568	520	2428	2180
2	2519	2536	1145	1161	2766	2499
1	2879	2768	1739	1819	2768	2763
0.5	2862	2924	2081	2062	2762	2768
0.25	2851	2959	1942	1847	2910	2834
0.13	2839	2690	1987	1932	2990	2694

Exhibit 1
Note 3, p. 5

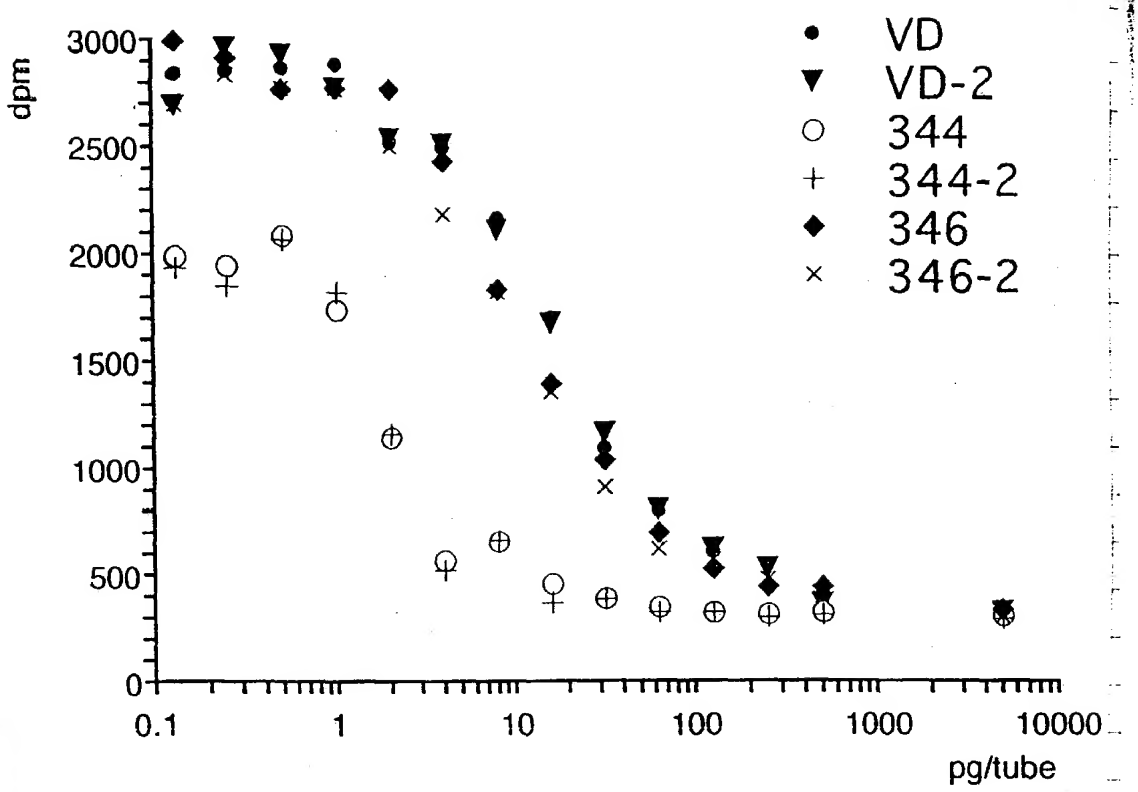
0	85 2744	86 2982	87 3149	88 3048	2980
blank	89 224	90 166	91 174	92 311	218
total count	93 7965	94 8280	95 8052	96 8325	8155
入射量	97 16184	98 15926	99 16360	100 16561	16257
blank	101 27	102 59	103 43	104 34	40

(2762)

すべての実験値から218をひいて (2980−218)で
割り ×100して Bound[%]をだいさん

50
50+500+200

#B7



	pg/tube	VD	VD-2	344	344-2	346	346-2
0	5000.0	290.00	325.00	308.000	296.00	338.00	305.00
1	500.00	357.00	363.00	325.000	312.00	445.00	386.00
2	250.00	444.00	529.00	318.000	302.00	445.00	477.00
3	125.00	608.00	623.00	326.000	324.00	528.00	573.00
4	63.000	802.00	806.00	349.000	326.00	698.00	623.00
5	32.000	1094.0	1166.0	391.000	387.00	1041.0	913.00
6	16.000	1701.0	1676.0	458.000	369.00	1395.0	1357.0
7	8.0000	2164.0	2109.0	658.000	663.00	1834.0	1822.0
8	4.0000	2494.0	2511.0	568.000	520.00	2428.0	2180.0
9	2.0000	2519.0	2536.0	1145.00	1161.0	2766.0	2499.0
10	1.0000	2879.0	2768.0	1739.00	1819.0	2768.0	2763.0
11	0.50000	2862.0	2924.0	2081.00	2062.0	2762.0	2768.0
12	0.25000	2851.0	2959.0	1942.00	1847.0	2910.0	2834.0
13	0.13000	2839.0	2690.0	1987.00	1932.0	2990.0	2694.0

dpm

#B2 88% 9/12 B7 80%
#B3 84% 9/19 9/30
#B4 84% 9/21

〈結果〉

$$blank = \frac{224+166+174+311}{4} = 218$$

$$0 = \frac{2744+2982+3149+3048}{4} = 2980$$

coldの
すべての実験値から blankの平均値 218を引いて
drug 0のときの 平均 2980から 218をひいたもの
(2980−218 = 2762)で除し 100をかけ 結合率を
計算した.

$$total\ count = \frac{7965+8280+8052+8325}{4} = 8155\ dpm$$

8155/60 dps = 136 Bq　　800 μl 中 500 μlとってcountしたので"
136 × 8/5 = 217 Bq

11.4 GBq/mg なので　19 pg/tube

入れた量の平均は 16257 dpm であるので
271 Bq より
24 pg/tube

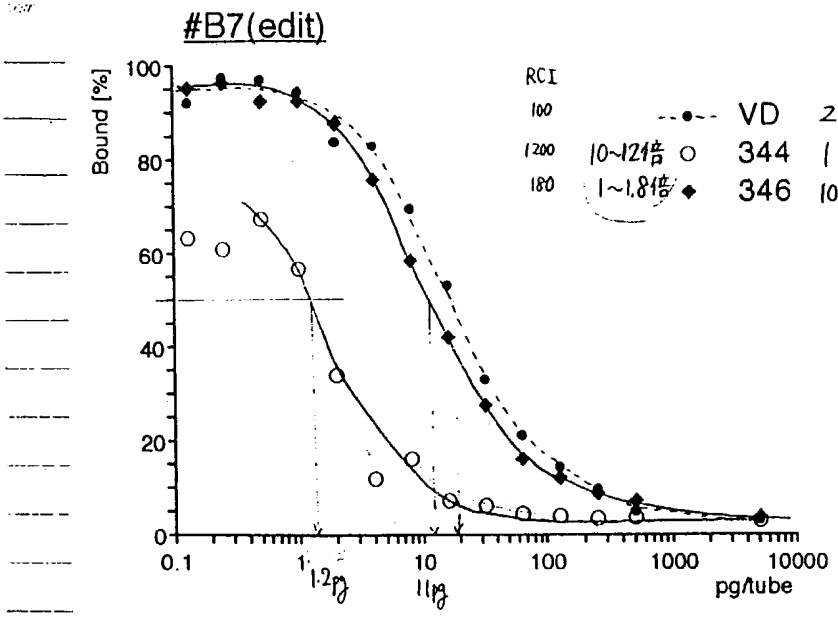
80%ぐらいが溶液中に存在し
あとはガラス壁等に吸着していると考えられる.

217 Bq/tube = 217/4.85T mol/(50+500+50) μl
= 0.075 nM

又は 1009 25日にとしてなた ここにこわれたものの countかもしれない

Exhibit 1
Note 3, p. 8

Bovine
Chicken



pg/tube	VD	VD-2	VD-	344	344-2	344-	346	346-2	346-
5000.0	2.6068	3.8740	3.2404	3.25851	2.8240	3.0413	4.3447	3.1499	3.7473
500.00	5.0326	5.2498	5.1412	3.87400	3.4033	3.6387	8.2187	6.0825	7.1506
250.00	8.1825	11.260	9.7212	3.62056	3.0413	3.3309	8.2187	9.3773	8.7980
125.00	14.120	14.663	14.392	3.91021	3.8378	3.8740	11.224	12.853	12.038
63.000	21.144	21.289	21.217	4.74294	3.9102	4.3266	17.379	14.663	16.021
32.000	31.716	34.323	33.020	6.26358	6.1188	6.1912	29.797	25.163	27.480
16.000	53.693	52.788	53.240	8.68936	5.4671	7.0782	42.614	41.238	41.926
8.0000	70.456	68.465	69.461	15.9305	16.112	16.021	58.508	58.074	58.291
4.0000	82.404	83.020	82.712	12.6720	10.934	11.803	80.014	71.035	75.525
2.0000	83.309	83.925	83.617	33.5626	34.142	33.852	92.252	82.585	87.419
1.0000	96.343	92.324	94.334	55.0688	57.965	56.517	92.324	92.143	92.234
0.50000	95.728	97.972	96.850	67.4511	66.763	67.107	92.107	92.324	92.216
0.25000	95.329	99.240	97.285	62.4185	58.979	60.699	97.466	94.714	96.090
0.13000	94.895	89.500	92.198	64.0478	62.056	63.052	100.36	89.645	95.004

#B7

/ / / 70ØA

2min

RY NO. 2: [H-3 DPM ESCR 2min 15:07

CYCLE : 1

[1] PRESET TIME (Min.) 2.0
[2] REPEAT 1
[3] CYCLE 1
[4] DATA DPM
[5] ISOTOPE H
[6] B.K.G SUB NO
[7] HEAD PRINT YES

* FUNCTION MODE *

[1] STANDARDIZATION ESCR
[2] CURVE AUTO
[3] REJECT NO
[4] ESCR PRESET TIME (Min.) 0.4
[5] CONSTANT RATIO NO
[6] CLEAR CHECK NO
[7] 2% ERROR NO
[8] FORMATTING NO
[9] FILE NO
[10] REPEAT REPLICATE NO
[11] AWS YES
[12] QUENCHING LEVEL AUTO
[13] BECQUEREL NO
[14] HALF LIFE NO
[15] CALCULATION NO
[16] HISTOGRAM NO

CURVE NO. = 3

EFF. LOW ENERGY Q:N A= -0.00789 B= 0.41072 C= 0.45704 D=-124.77292
EFF LOW ENERGY Q:H A= 0.00660 B= 0.20210 C= 0.42623 D= -2.11626

NO	ESCR	TIME	H-CPM	H-DPM	H-EFF
1	26.26	2.0	80.5	290.6	27.70
2	26.18	2.0	97.5	357.9	27.24
3	26.20	2.0	121.5	444.1	27.36
4	26.24	2.0	168.0	608.9	27.59
5	26.22	2.0	220.5	802.6	27.47
6	26.20	2.0	299.5	1094.7	27.36
7	26.22	2.0	467.5	1701.6	27.47
8	26.26	2.0	599.5	2164.0	27.70
9	26.20	2.0	682.5	2494.5	27.36
10	26.24	2.0	695.0	2519.1	27.59
11	26.24	2.0	794.5	2879.8	27.59
12	26.26	2.0	793.0	2862.5	27.70
13	26.26	2.0	790.0	2851.6	27.70
14	26.18	2.0	773.5	2839.1	27.24
15	26.22	2.0	89.5	325.8	27.47
16	26.20	2.0	99.5	363.7	27.36
17	26.22	2.0	145.5	529.6	27.47
18	26.20	2.0	170.5	623.2	27.36
19	26.24	2.0	222.5	806.5	27.59
20	26.22	2.0	320.5	1166.5	27.47
21	26.24	2.0	462.5	1676.4	27.59
22	26.22	2.0	579.5	2109.2	27.47
23	26.20	2.0	687.0	2511.0	27.36
24	26.22	2.0	697.0	2536.9	27.47
25	26.22	2.0	760.5	2768.0	27.47
26	26.22	2.0	803.5	2924.5	27.47
27	26.22	2.0	813.0	2959.1	27.47
28	26.28	2.0	748.5	2690.7	27.82
29	26.20	2.0	84.5	308.8	27.36
30	26.22	2.0	89.5	325.8	27.47
31	26.22	2.0	87.5	316.5	27.47
32	26.26	2.0	90.5	326.7	27.70
33	26.24	2.0	96.5	349.8	27.59
34	26.24	2.0	108.0	391.5	27.59
35	26.20	2.0	125.5	458.7	27.36
36	26.26	2.0	182.5	658.8	27.70
37	26.20	2.0	155.5	568.4	27.36
38	26.20	2.0	313.5	1145.8	27.36
39	26.24	2.0	480.0	1739.8	27.59
40	26.22	2.0	572.0	2081.9	27.47
41	26.24	2.0	536.0	1942.8	27.59
42	26.22	2.0	546.0	1987.3	27.47
43	26.20	2.0	81.0	296.1	27.36
44	26.28	2.0	87.0	312.8	27.82
45	26.24	2.0	83.5	302.7	27.59
46	26.24	2.0	89.5	324.4	27.59
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Exhibit 1
Chart 3, p. 1

21	26.24	2.0	462.5	1676.4	27.59
22	26.22	2.0	579.5	2109.2	27.47
[illegible]	[illegible]	2.0	667.0	2311.0	27.36
[illegible]	[illegible]	2.0	697.0	2536.9	27.47
25	26.22	2.0	760.5	2768.0	27.47
26	26.22	2.0	803.5	2924.8	27.47
27	26.22	2.0	813.0	2959.1	27.47
28	26.26	2.0	748.5	2690.7	27.82
29	26.20	2.0	84.5	306.8	27.36
30	26.22	2.0	89.5	325.8	27.47
31	26.22	2.0	87.5	318.3	27.47
32	26.26	2.0	90.5	326.7	27.70
33	26.24	2.0	96.5	349.8	27.59
34	26.24	2.0	108.0	391.5	27.59
35	26.20	2.0	125.5	458.7	27.36
36	26.26	2.0	182.5	658.9	27.70
37	26.20	2.0	155.5	568.4	27.36
38	26.20	2.0	313.5	1145.8	27.36
39	26.24	2.0	480.0	1739.8	27.59
40	26.22	2.0	572.0	2081.9	27.47
41	26.24	2.0	536.0	1942.8	27.59
42	26.22	2.0	546.0	1987.3	27.47
43	26.20	2.0	81.0	296.1	27.36
44	26.38	2.0	87.0	312.8	27.82
45	26.24	2.0	83.5	302.7	27.59
46	26.24	2.0	89.5	324.4	27.59
47	26.24	2.0	90.0	326.2	27.59
48	26.24	2.0	107.0	387.8	27.59
49	26.24	2.0	102.0	369.7	27.59
50	26.24	2.0	183.0	663.3	27.59
51	26.24	2.0	143.5	520.1	27.59
52	26.22	2.0	319.0	1161.1	27.47
53	26.24	2.0	502.0	1819.6	27.59
54	26.24	2.0	569.0	2062.4	27.59
55	26.22	2.0	507.5	1847.2	27.47
56	26.22	2.0	531.0	1932.7	27.47
57	26.20	2.0	92.5	338.1	27.36
58	26.20	2.0	122.0	445.9	27.36
59	26.26	2.0	123.5	445.8	27.70
60	26.20	2.0	144.5	528.1	27.36
61	26.22	2.0	192.0	698.8	27.47
62	26.22	2.0	286.0	1041.0	27.47
63	26.24	2.0	385.0	1395.5	27.59
64	26.24	2.0	506.0	1834.1	27.59
65	26.18	2.0	661.5	2428.0	27.24
66	26.22	2.0	760.0	2766.2	27.47
67	26.20	2.0	757.5	2768.7	27.36
68	26.22	2.0	759.0	2762.6	27.47
69	26.22	2.0	799.5	2910.0	27.47
70	26.22	2.0	821.5	2990.0	27.47
71	26.26	2.0	84.5	305.0	27.70
72	26.24	2.0	106.5	386.0	27.59
73	26.18	2.0	130.0	477.2	27.24
74	26.22	2.0	157.5	573.3	27.47
75	26.20	2.0	170.5	623.2	27.36
76	26.22	2.0	251.0	913.6	27.47
77	26.22	2.0	373.0	1357.6	27.47
78	26.20	2.0	498.5	1822.0	27.36
79	26.26	2.0	604.0	2180.2	27.70
80	26.16	2.0	678.0	2499.1	27.13
81	26.20	2.0	756.0	2763.2	27.36
82	26.22	2.0	760.5	2768.0	27.47
83	26.20	2.0	775.5	2834.5	27.36
84	26.18	2.0	734.0	2694.1	27.24
85	26.22	2.0	754.0	2744.4	27.47
86	26.22	2.0	819.5	2982.8	27.47
87	26.24	2.0	869.0	3149.8	27.59
88	26.22	2.0	837.5	3048.3	27.47
89	26.28	2.0	62.5	224.7	27.82
90	26.30	2.0	46.5	166.5	27.93
91	26.32	2.0	49.0	174.7	28.05
92	26.30	2.0	87.0	311.5	27.93
93	26.24	2.0	2197.5	7965.1	27.59
94	26.26	2.0	2294.0	8280.6	27.70
95	26.22	2.0	2212.5	8052.9	27.47
96	26.24	2.0	2297.0	8325.8	27.59
97	27.46	2.0	5542.0	16184.1	34.24
98	27.52	2.0	5503.0	15926.2	34.55
99	27.52	2.0	5653.0	16360.3	34.55
100	27.56	2.0	5756.5	16561.4	34.76
101	27.64	2.0	9.5	27.0	35.17
102	27.62	2.0	21.0	59.9	35.07
103	27.58	2.0	15.0	43.0	34.96
104	27.60	2.0	12.0	34.3	34.96